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A Study of Normal Operant Movement of a Diarthrodial Joint

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A STUDY OF NORMAL OPERANT MOVEMENT OF A DIARTHRODIAL JOINT

BY

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AUTOBIOGRAPHY

James A. Coglianese was born in Chicago, Illinois on October 6, 1942. He attended both elementary and high school in the city of Chicago.

In September, 1960 he entered the University of Notre Dame, Notre Dame, Indiana. He received the degree of Bachelor of Science in Pre-Professional Studies in June, 1964.

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CHAPTER I

INTRODUCTION AND STATEMENT OF PROBLEM

The chick embryo quadrate-quadratojugal articulation is a bilateral synovial lined diarthrodial joint with an interposing disc. The study of this articulation is undertaken in this investigation because it is comparable to the human temporal mandibular joint which is also a bilateral synovial lined diarthrosis with an interposing disc. The study of the functional development of such diarthrodial joints is essential because of the apparent lack of specific theoretical information on which to base the therapeutic management of mandibular joint disorders. This lack of information cannot be rectified through clinical investigations alone, basic research is required as well. It is essential, therefore, that this study of the normal operant movement of the chick embryo quadrate-quadratojugal articulation be carried out so that any subsequent investigations on the experimental embryogenesis of this articulation will have a sound basis upon which to evaluate their findings.

It is the purpose of this study to note the normal operant movement of the White Leghorn chick embryo

quadrate-quadratojugal articulation. Evidence of articular movement is noted by studying the rate of beak movement. Therefore, to determine the rate of beak movement as an index for articular movement, an accurate reproducible methodology is developed for the actual counting of the number of normal opening and closing movements of the beak while being studied under *in vivo* conditions.

CHAPTER II

REVIEW OF THE LITERATURE

A. Basic Chick Embryology

Lillie (1927) considered the development of the skull in two sections; first, the dorsal division associated with the brain and sense organs (neurocranium), and second, the visceral division or splanchnocranium. He noted that chondrification begins in the neurocranium about the sixth day and appears first near the midline and extends out laterally. By the eighth day, the entire neurocranium forms a continuous mass of cartilage. On the origin of the splanchnocranium, he stated that two skeletal elements arise in the mandibular arch on each side, a proximal one (the palato-quadrate) and a distal one (Meckel's cartilage). The former is relatively compressed, and the latter an elongated element (Fig.1). The palato-quadrate soon develops a triradiate form of three processes, one of which, the processus articularis, furnishes the articulation for the mandibular arch. It consists of two rods of cartilage in the rami of the mandibular arch, which articulate proximally with the processus articularis of the palato-quadrate cartilage, and meet distally at the symphysis

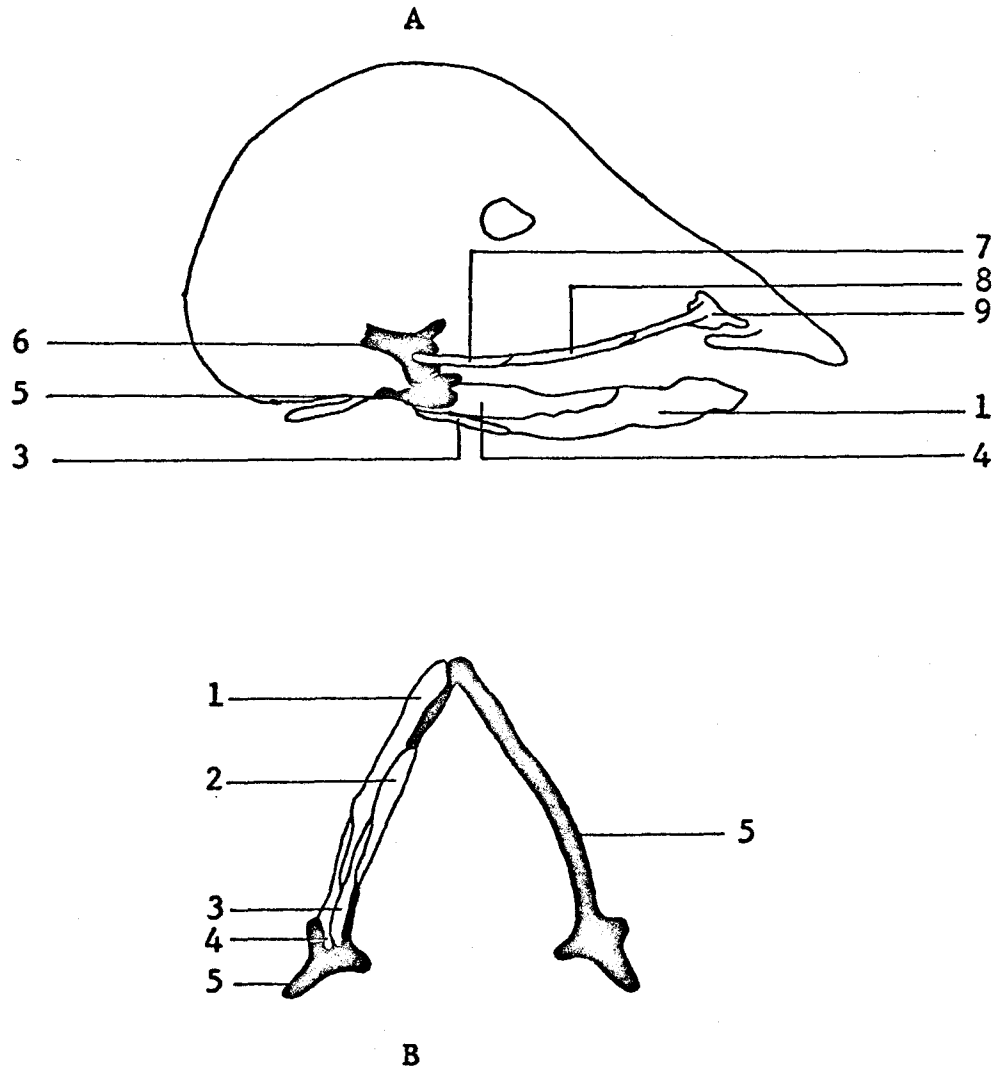

Fig. 1. A - Skull of a chick embryo 65 mm. length; Cartilage bones shaded, Membrane bones non-shaded. B - Inferior view of Mandibular Arch. 1. Dentale, 2. Operculare, 3. Angulare, 4. Supra-angulare, 5. Meckel's cartilage, 6. Quadrate, 7. Quadratojugal, 8. Jugal, 9. Maxilla. (Drawn from Lillie, Development of the Chick, p.430)

of the mandibular arch. The form of the articulation of the mandibular arch is defined in the cartilage at seven to eight days.

In considering the ossification of the skull, Lillie noted that both endochondral and membranous bones are present. The cartilage bones of interest are: a) the palato-quadrate cartilage which furnishes the quadrate bone, and b) the articulare which arises in Meckel's cartilage and later fuses with the membrane bones. The membrane bones of interest are the: a) maxillae, b) jugals, c) quadratojugals, and d) bones surrounding Meckel's cartilage and forming the part of the mandibular arch: angulare, supra-angulare, operculare, and dentale (Fig.1). The embryonic chick's skull is characterized by a wealth of distinct bones, but in the course of development many of these fuse completely. In order of development, the membrane bones precede the cartilage bones. At the beginning of the tenth day, the following bones are present in the form of delicate reticulated bars and plates: all four bones of the mandible, the central part of the maxilla, and the jugal and quadratojugal. A faint band of perichondral bone is beginning to appear around the otic process of the quadrate, the first cartilage bones to show any trace of ossification. These ossifications appear practically simultaneously. It should be

noted, therefore, that at the beginning of the tenth day, all the associated structures of the quadrate-quadratojugal articulation are present and are ready for maturation.

Hamberger and Hamilton (1951) categorized a series of normal stages in the development of the chick embryo which covers the entire period of incubation. They noted that there are many factors responsible for the lack of correlation between chronological and structural age. Among those listed were: genetic differences in the rate of development of different breeds (eg. the White Leghorn embryo develops rapidly and may hatch approximately a day earlier than other breeds), seasonal differences in the viability and vigor of embryos, differences in the stage of development when incubation is started, differences in the lapse of time between laying and incubation, differences in the temperature of eggs when placed in the incubator and in the size of individual eggs, and differences in the temperature of incubation and size of the incubator. Instead of chronologic age, the authors staged the embryos on the basis of external characteristics. Other authors have described chick embryology (Patten, 1929; Jacobson, 1938; and Freeman et al. 1964) but none have so aptly described it from an observational-anatomic viewpoint. Hamberger and Hamilton's description of the development of the visceral arches is compatable with Lillie's description (1927). The embryos

used in the study were of several breeds: White Leghorn, Barred Plymouth Rock, and Rhode Island Red. These were incubated at 37.5°C, sacrificed at particular stages, and were fixed in Bouin's solution or formalin.

Hamberger (1956) published a drawing of a 9 day old chick embryo in ovo which demonstrates all the structures pertinent to this study (Fig.2). It should be noted that the illustration is quite diagramatic, but of particular importance is the close proximation of the chorio-allantoic membrane, with its contained allantoic blood vessels, to the shell membrane. In addition, the shell membrane can be seen to divide at the larger end of the egg so as to allow the presence of an air chamber within the egg but outside of the inner shell membrane.

B. Motility of the Chick Embryo

Kuo (1932) presented his findings on the chronology and general nature of the behavior of the chick embryo. The observational technique was used to note embryonic development of the chick within the egg. The egg shell and outer egg shell membrane were removed at the air chamber end of the egg. Melted vaseline was then applied to the inner egg shell membrane so as to render the membrane transparent and to allow the observer to note embryonic changes. The eggs were incubated at temperatures between 102°F and 104°F. The operated end of

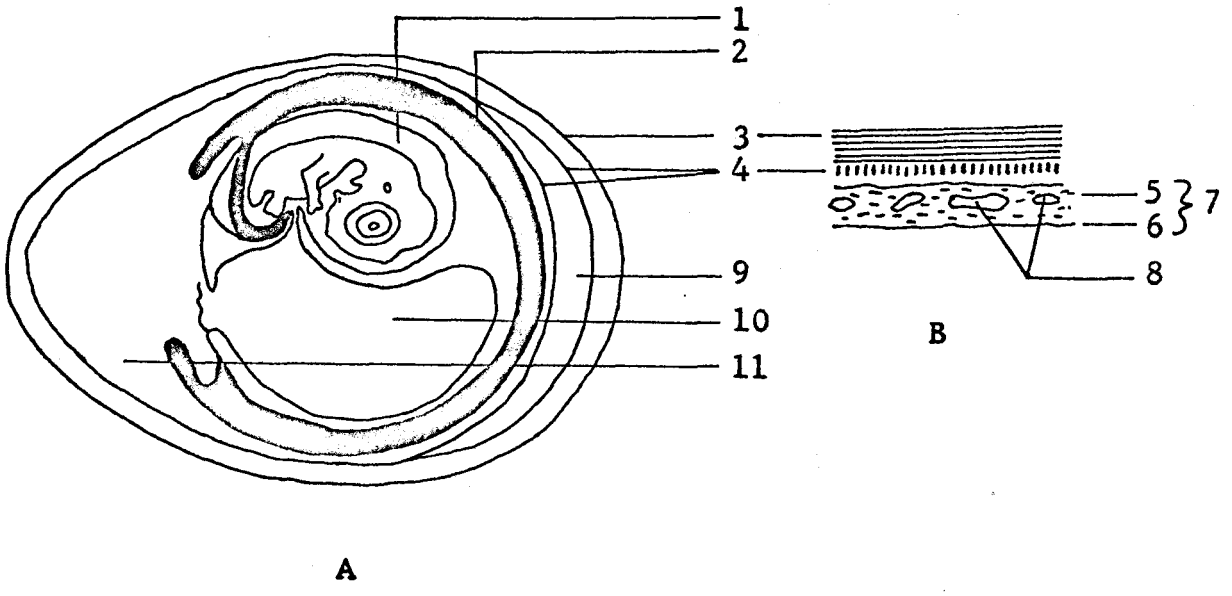

Fig.2. A - Diagram of chick embryo in ovo at 9 days incubation. B - Enlarged part of A. 1. Amniotic Cavity, 2. Allantois, 3. Shell, 4. Shell Membrane, 5. Chorion, 6. Allantoic Wall, 7. Chorio-allantoic Membrane, 8. Allantoic Blood Vessels, 9. Air Chamber, 10. Yolk Sac, 11. Albumen. (Drawn from Hamberger, Manual of Experimental Embryology, 1956.)

the egg was tightly covered with a portion of old egg shell so as to prevent excessive evaporation. During observation, the incubator was opened and the egg shell cover removed. As incubation proceeded, the size of the air chamber increased as did the size of the embryo. It became necessary, therefore, to remove more egg shell and coat an additional portion of the inner shell membrane with melted vaseline. This was necessitated by the fact that the initial opening of the egg was governed by the reduced size of the air chamber present in the eggs operated at 3 days incubation. Embryos were observed once a day from 3 days to hatching with the light source being a strong microscopic lamp. Breeds of eggs were White Leghorn with others from mixed Chinese breeds slightly smaller in size than the White Leghorn.

Kuo presented the chronologic development of behavior of the chick by studying thirty-nine different types of movements including heart beat, head lifting and bending, appendage movement, body turning, respiration, and hatching. He noted that bill clapping (the rapid opening and closing of the beak) occured at day 9 in 14.5% of the embryos, at day 10 in 39.1% of the embryos, at day 11 in 33.6% of the embryos, and at day 12 in 12.6% of the embryos tested. Data for beak movement was given, but such data is not pertinent to this study for included in the totals were not only bill clapping, but also

so-called mouth movements occuring on day 5, opening or closing movements but not necessarily both, and uplifting and forward thrusting movements of the beak after the protrusion of the neck during piercing of the membranes in hatching. Kuo noted that successive movements of the chick embryo are directed cephalo-caudally in that head movements appear first, then trunk movements, then movements of the extremities, and finally tail movement. In discussing the embryo's response to light, Kuo stated that such response is not noted until the end of the 17th day. In response to sound or vibration, practically every part of the body is involved where the body wriggles, wings and legs move, and the eyes and beak open at the same time. However, in response to light, the embryo may only open or close its eyes without involving movements of other body parts.

In a later article by Kuo in the same year (1932), he discussed the mechanical factors in the various stages leading to hatching. In order to be properly situated for hatching, the embryo must pass through the following critical stages: a) orientation of the embryo, b) torsion and flexion, c) lying of the embryo at the large end of the egg at 8-9 days, d) fixation of body position where the embryo lies with its back on the yolk sac and its body axis at right angle to the long axis of the egg at 8-12 days, e) changes in positional

relation between the embryo and yolk sac where the yolk sac comes over to cover the ventral side of the embryo, f) turning of the body so as to lie lengthwise in the egg, and g) protrusion of the neck into the air chamber under the membranes.

Windel et al. (1934) discussed the appearance of somatic movements in the chick embryo from day 4-9. Eggs were incubated in an electric oven at temperatures of 35° - 37°C. maintained by electric light bulbs. Eggs were opened as described by Kuo (1932), however, attempts to leave the inner shell membrane intact and to render it transparent with vaseline were unsuccessful. Indeed, the opacity of the membrane could not be overcome sufficiently so that only gross movements were observable and fine details imperceptible. Therefore, Windel et al. removed the inner shell membrane. Their results were to confirm, in a general way, Kuo's (1932) findings as to the order of appearance of the spontaneous activity of the chick embryo.

Hamberger et al. (1963) discussed observations and experiments on spontaneous rhythmical behavior in the chick embryo. White Leghorn embryos were prepared by sawing a large window in the shell. Incubation was at 37.5°C. in a plastic box with two circular windows to give access to the preparation. Observations were made 2-3 hours after opening, and the embryos were staged according to Hamberger and Hamilton stage series

(1951). Sectors of the spinal cord were coagulated with an electrocautery needle and then removed. Extirpations were made at either the cervical, brachial, or thoracic level for the purpose of studying motor activity of isolated parts with those of normal or unoperated embryos. Appendage, tail, trunk, and general body movement were the criteria for motility rather than beak or clapping movements. Embryos from 3½ to 10 days were observed, and during this period, all motility is self generated. Extirpation of segments of spinal cord did not preclude motility in isolated parts. Hence, parts of the spinal cord are capable of causing autonomous motility. Early behavior of normal embryos up to 9 to 10 days is rhythmical; activity phases of 5-15 seconds are followed by inactivity phases lasting for 30-60 seconds. The activity and inactivity phases of isolated parts were more regular and had longer periods of activity and inactivity when compared to the normal embryos. It was suggested, then, that the motility of normal embryos results from a superimposition of a shorter cycle originating in the brain over a longer cycle intrinsic in the spinal cord.

Hamberger et al. (1965) studied the periodic motility of normal and spinal chick embryos to 19 days. Materials and methods were the same as in the 1963 study. It should be stated again that overall chick embryo motility was the criteria for

determining activity or inactivity during a 15 minute observation period. The percentage of time spent in activity rises steadily from less than 10% at the beginning of motility at 3½ days to 80% at day 13. This peak is maintained up to day 17; subsequently motility declines. This activity is spontaneous in that it is not reflexogenic. The motility of the chick embryo is uncoordinated from day 6½ on. During an activity phase, all parts that are capable of motility at a given stage move in an unintegrated, unpredictable random fashion. The spinal embryos retained their capacity for cyclic motility, however, it is reduced by 10-20% at all stages between the 8th and the 17th day. This fact apparently disputes earlier findings on the effect of the brain on the spinal cord, for in this study brain influence lengthened the duration of the activity phase.

Hamberger et al. (1966) continued his series of articles by discussing the motility in the chick embryo in the absence of sensory input. Incubation and preparation of the embryos was as described by Hamberger in 1963. Complete deafferentation of the leg level was accomplished in the 2-day embryos by extirpation of lumbar spinal cord, with simultaneous extirpation of the entire spinal cord in the thoracic level to exclude sensory input from more rostral levels. In control embryos, only the thoracic gap was made. Recordings of leg

motility were made at 8½, 11, 13, 15, and 17 days. Overall activity and the duration of activity and inactivity phases were found to be the same in experimental embryos and in control embryos with thoracic gaps. It was concluded that sensory input is not necessary for the triggering, maintainance, or periodicity of leg motility.

Hamberger et al. (1967) discussed prehatch motility and hatching behavior of chick embryos. He used an inbred stock of a white breed stock Hyline No. 934. Incubation and preparation of embryos was as described by Hamberger (1963), however, no spinal segments were denervated and only so called normal embryos were studied. He noted that coordinated movements appear to originate around day 17 which do not seem to be related to the random movements characteristic of earlier embryos. The random movements continue until the onset of hatching, but they are suspended temporarily while the coordinated movements are performed. Such coordinated movements include: 1) the lifting of the head out of the yolk sac and the tucking of the right side of the head under the right wing at day 18, 2) the first cracking of the shell at day 20, and 3) the actual hatching. The head movements are a part of these coordinated movements during the final days of incubation. Hamberger recorded the mean number of beak claps from day 15 to hatching. If the beak was buried in the yolk

sac or covered by the wing, a small opening was made in the membranes and the beak exposed. The recording was made 30 minutes after exposure of the beak. The mean number of beak claps for both the unoperated embryos and operated embryos was reported in one total. The following findings were reported for the Hyline No. 934 breed: days 15-19 demonstrated mean rates of 13 per minute, and day 20, on which the embryos hatched, demonstrated a mean rate of 28 per minute.

Corner et al. (1967) studied developmental patterns in the central nervous system of the chick embryo. Corner attached electrode leads to the embryos to record EEG, ECG, and EMG values during periods of somatic motility. His findings complement those of Hamberger in that motility is stage specific. He disputed some of the findings of Kuo (1932) on the basis that Kuo's procedure of using melted vaseline applied to the shell membrane reduced oxygen consumption of the egg up to 40%.

Kovach (1968) discussed the spatial orientation of White Rock and California White chick embryos. Eggs were incubated while placed on an automatic turner which rotated the eggs 90° every 4 hours. Eggs were transferred to a stationary incubator for the last five days. The first opening of the eggshell upon hatching appeared on the upper surface of the egg in 93% of the hatchings. Spatial orientation of the embryo was related to the air chamber location. Kovach disputed

Hamberger's random vs. coordinated movement theory by stating that the appearance of the first opening on the upper surface of the hatching egg is determined by density distribution and passive gravitational turning of the egg and embryo and possibly by additional behavioral factors. The latter may imply a sensory capacity for the recognition of the upward position and behavioral adjustment of this position in chick embryos 17 days and older. Kovach further criticized the methods employed by Kuo (1932) and Hamberger et al. (1966) for the study of the embryo motility in which a part of the egg shell was removed and embryonic behavior observed directly. He stated that this procedure is not satisfactory because such methods disturb the pressure balance between the shell, air chamber, and embryo, and may influence the activity of the embryo.

Soudah (1969) studied the relation between curare-like derivatives and the quadrate-quadratojugal articulation in White Leghorn chick embryos. Preparation of the embryos and incubation were as indicated by Hamberger (1963). Curare-like derivatives were infused through the allantoic blood vessels to cause immobilization of the quadrate-quadratojugal articulation from day 7 to hatching. Fig. 3 is a photomicrograph from that study. It illustrates an anterior-posterior section through the articulation of a normal embryo

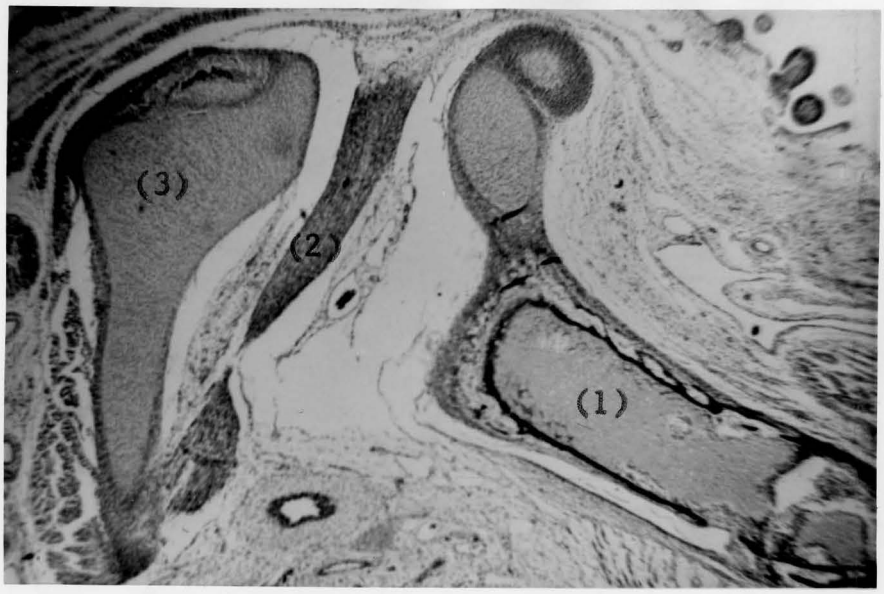

Fig. 3. A photomicrograph of an anterior-posterior histologic section of a normal quadrate-quadratojugal articulation of a White Leghorn chick one hour post-hatching. 1. Quadratojugal, 2. Articular Disc, 3. Quadrate.

one hour post hatching. The findings of Soudah's study of the experimental embryogenesis of the articulation were recorded post-hatching and such results are, at this time, still forthcoming.

C. Experimental Environment Effect on the Chick Embryo

Hamilton (1952) studied the sensitive periods during development of the chick embryo. Observations were based on percentage hatching and mortality of unoperated normal embryos subjected to a variety of environmental conditions. He stated that temperature is the most obvious physical factor affecting development, since hatching does not occur above 39.5° C. or below 35.5° C. The optimum temperature for development seems to be 37.8° C. (100° F.). Temperatures above or below the optimum accelerate or retard the rate of development, respectively, up to 10-12 days. After 10-12 days, variations in temperature in either direction are growth retarding. Closely related to temperature is humidity, since effects of one may be modified by a change in the other. Optimum humidity was given as 60% at 38° C. If the humidity is too low, the egg loses too much water and growth is retarded. When the humidity is too high, the chorio-allantoic membrane fails to dry properly so that the embryo drowns in its own fluids. A third physical factor is gravity. Here

Hamilton supports the findings of Kuo (1932).

Drachman and Sokoloff (1966) discussed the role of movement in embryonic development. They used the White Leghorn chick embryo as the test animal and studied joint formation during induced experimental joint paralysis. It should be noted that this investigation did not include study of the quadrate-quadratojugal articulation. Experimental paralysis was started at 6-7 days and was produced by three different procedures: 1) by the continuous infusion of decamethonium from a syringe activated by a constant rate infusion pump with the drug being delivered through a polyethylene microcatheter secured within a chorioallantoic vessel of the embryo, 2) by repeated intravenous injection of botulinim toxin, and 3) by extirpation of the lumbo-sacral spinal cord. Striking abnormalities of joint development were found which were identical in the embryos paralyzed by the three techniques. Articular cavities failed to develop in the embryos' paralyzed joints, although the preparatory changes in the cellular architecture of the joint regions proceeded otherwise normally. Fusion occured across the presumptive joints, first by loose fibrovascular tissue, and later by compact fibrous connective tissue, cartilage, or bone. The shape of the articular surfaces lacked fine sculptural details. Certain accessory articular structures,

which are important to the mechanical function of the joints, failed to develop. These include some sesamoids, and all adventitious cartilages, and intra-articular ligaments. Certain skeletal prominences which normally give attachment to muscles were absent or distorted in the paralyzed embryos. Drachman felt that these malformations applied to joints and bones of all types in many different sites in immobilized embryos.

Tamimie et al. (1967) studied the effect of continuous and intermittant light exposure on the embryonic development of chicken eggs. Unoperated, normal White Leghorn embryos, incubated at standard temperature and humidity, were subjected to a 100 watt incandescent bulb from 0 to 21 days. Light was either continuous for this period or interrupted at one second intervals. His findings indicated a high percentage of dead embryos, delay in hatching time, malformed feet, legs, eyes, and mandibles. The severity of this effect was more pronounced with continuous rather than intermittant light.

Gimeno et al. (1967) studied the acceleration of heart rate by visible light in the 2-5 day chick embryo. Hearts were dissected from the embryos and freely suspended in a phosphate-buffered medium at 37^{o} C. Light appeared to stimulate the embryonic hearts suggesting: 1) that there is a substance (or substances) present in the chick embryo heart

capable of absorbing light energy and of transferring this energy into mechanical contraction, or 2) that such stimulation may be related to the synthesis or release of acetylcholine. Whatever the mechanism, such stimulation would effect developmental growth of the embryo.

CHAPTER III

MATERIALS AND METHODS

A. Care of the Chick Embryo Up to Day Seven

Forty-eight White Leghorn chick embryos were obtained from the C.L. Sharp Hatchery of Glen Ellyn, Illinois. They were placed in a 155 watt electric incubator manufactured by Brower Manufacturing Company, Quincy, Illinois, which was obtained through Sears Roebuck and Company Farm Catalog Sales in Chicago, Illinois. Incubation of the fertile eggs was initiated on the day of collection. The horizontal metal disc or ceiling of the incubator, from which the thermostat and heating element were suspended, was removed through the use of a metal cutting Sabre saw. One fourth inch clear Plexiglas was cut to the demensions of the opening, placed on the top of the incubator, and was sealed with window putty. The thermostat and heating element were removed from the metal disc and were attached to the new Plexiglas ceiling. This procedure allowed clear visibility to all parts of the incubator without necessitating the opening of the incubator. Maintainance of even incubation temperature with the Plexiglas ceiling was equal to that of the metal ceiling. A thermometer

was placed on the raised wire floor of the incubator at the level of the eggs, and a constant temperature of 36° C. was maintained. Below the wire floor, a water pan maintained the humidity at 60% as indexed by a humidity indicator housed within the incubator. Eggs were rotated 180° once every twelve hours up to day 7 at which time they were operated upon.

The fertile eggs were exposed to 12 hours of light and 12 hours of dark. The source of light was from two 7½ watt white electric bulbs attached by their bases to the Plexiglas ceiling. The bulbs were placed so that an even distribution of light was obtained throughout the incubator. The bulbs were reduced to half their brightness by an Ohmite vitreous enameled rheostat (25 watt, Model H, Series A, 1000 ohms) obtained through Allied Radio Corporation Industrial Catalog Sales of Chicago, Illinois. Light was just sufficient so an adequate observation of the embryo in ovo was possible. The heat generated by the rheostat and lights, both of which being attached to the Plexiglas ceiling, did not affect the incubation temperature due to the automatic temperature control afforded by the Plexiglas mounted thermostat. The 12 hour on - 12 hour off cycle of the lights was controlled electrically by a T 1975 Intermatic Skipper Program Timer manufactured by the International Register Company and

obtained through Allied Radio Corporation Industrial Catalog Sales of Chicago, Illinois. Such lighting conditions, temperature, and humidity were maintained as stated here throughout the entire incubation period of the embryo.

The incubator was placed in a darkened, windowless room away from any drafts. A black, matt surface, cardboard cover with air holes was placed over the Plexiglas at all times except for during observation. All observations were made with the room darkened so that the only light entering the incubator was from the light bulbs within the incubator itself.

B. Preparation of the Embryos for Observation

On day 7, the eggs were candled with the use of the light projected through a home slide projector. The quantity of light was reduced by inserting an opaque cardboard slide with an opening cut in the center about the size of a dime. The exact location of the embryo was noted and marked on the shell. Non-fertile eggs were discarded. Fertile eggs were placed on egg holders and were placed back into the incubator. (Egg holders had been made by filling the bottom of small paper drinking cups to ¼ inch with dental stone which was allowed to harden. Later, the plaster discs were removed from the cups and two strips of window putty were placed near the periphery of the discs. The putty adhered to both the dried

plaster discs and the egg shell.)

Prior to shell opening, the entire work area was disinfected with Lysol Solution and all instruments were immersed in 70% alcohol for 20 minutes. The operator wore a surgical mask and the areas on the egg shell to be opened were swabbed with 70% alcohol and dried with sterile cotton. One of the points of a small watchmaker's forcepts was used to penetrate through to the air chamber. A 3/8 inch diamond disc on a dental handpiece was used to groove the egg shell without cutting all the way through to the shell membrane. If the shell were completely penetrated, possible rupture of the allantoic vessels would occur due to the close proximity of the chorio-allantoic and shell membranes, and possible death of the embryo would result. Three parallel grooves were cut in the shell above the embryo perpendicular to the long axis of the egg about one inch long and 3/8 inch apart. These grooves were joined down the center by a groove about 1½ inches long that paralled the long axis of the egg. An oval groove at the perimeter was then made which joined all the other grooves. This outlined the location of the future opening (about 1 X 1½ inches) and generally followed the contour of the egg. Any shell fragments or grindings were then removed by brushing the area with sterile cotton so that these would not contaminate the embryonic structures upon the

opening of the egg.

Watchmaker forcepts were then used to grasp one of the shell segments while being careful not to pierce the shell membrane with the forcepts. The shell segment was then gently removed in such a way that the shell membrane remained fixed to the shell segment allowing the separation of the shell membrane from the chorio-allantoic membrane. This separation was desirable since it broke the seal of the egg which allowed the embryo to drop down away from the operated site by occupying the space in the egg previously taken up by the air chamber. When the embryo dropped down, air was forced out of the air chamber through the hole punctured in the shell. Once the embryo had dropped, great care was taken to remove the rest of the shell segments one by one so that any chorio-allantoic membrane still adhering to the shell membrane at the periphery of the opening would not be traumatized.

Once the opening was complete, a pre-cut piece of plastic kitchen wrap (Saran Wrap) was placed over the opening. Four strips of ½ inch clear cellophane tape were used to seal down the plastic wrap to the egg shell, (Fig. 4.). Exposure of the embryo to the open air was minimized in that the elasped time from the dropping of the embryo to the placement of the plastic wrap was only one to two minutes. Fogging of the window occurred immediately but this completely disappeared

Fig. 4. The closed beak of a nineteen day White Leghorn chick embryo in ovo illustrating the transparent observation window.

a few minutes after the embryo was placed back into the incubator. It should be noted that the size of the window was proportional to the size of the air chamber into which the embryo dropped. Therefore, care was taken so as to make the window only as large as needed for observation in order not to crowd the embryo during the later stages of development. Operated eggs placed back into the incubator were separated so that vibrations occurring in one egg would not affect the motility in the neighboring egg. Care was taken to keep the embryonic membranes from touching the plastic wrap; for, if this occurred, the death of the embryo was inevitable. Operated eggs no longer were rotated or disturbed in any way for the remainder of the incubation period. All fertile were operated on day 7.

C. Method of Collecting Data

Observation of the embryos was initiated on the morning of the eighth day. The morning observation was begun one hour after the incubator lights were turned on so that any effect that the light might have had on the embryos would be minimized in the recordings. The evening observation was begun two hours before the incubator lights were turned off. The duration of the observation never exceeded two hours.

Beak clapping was observed where one count was defined as

an opening and closing of the beak regardless of amplitude. Such a movement would necessitate the contraction of both depressor and elevator muscles. An active opening with a subsequent relaxation of elevator muscles resulting in the passive closing of the beak was not counted as beak movement in this study. Beak movement was observed for a period of 15 seconds in each embryo. Beak movement caused a resultant vibration of the surrounding embryonic membranes as did other body motility. If there were no beak movement and no concurrent body motility, the surrounding embryonic membranes remained perfectly still. Therefore, in those embryos whose beaks were not visible at any time during the two hour observation period and whose surrounding embryonic membranes remained still for 15 seconds, a zero beak movement rate was recorded. In the greater majority of embryos whose position was such that the beak was observable at some time during the two hour observation period, recordings were made (0,1,2,3, etc.) only when the beak was completely visible for the entire 15 second interval. Therefore, any embryo whose beak was not visible for 15 seconds and whose surrounding embryonic membranes were not still for 15 seconds at any time during the two hour observation period, did not receive a recording for that observation period and were not included in this study.

Beak clapping was recorded by observation and the 15

second interval was determined by a micro-timer. A Series CM Recycling-Cam Foundation Unit (CM 6) and a Gear and Rack Assembly for a CM 6 (A 18), manufactured by Industrial Timer Corporation, Parsippany, New Jersey, was set for the 15 second interval correct to one fourth of a second. The timer was wired to a sound dampened 14-1 A.C. Type Push-Pull Solenoid, manufactured by Dormeyer Industries, Chicago, Illinois, which signaled the begining and the end of the 15 second interval. Both the timer and solenoid were obtained through Allied Radio Corporation Industrial Catalog Sales of Chicago, Illinois.

Rate of beak clapping was recorded at each of the two observation periods per day from day eight to hatching. The hatched chicks were allowed to live for forty eight hours to note normalcy at which time they were sacrificed.

Embryos were not staged according to Hamberger and Hamilton (1951) stage series for two reasons. First, observations of the embryos were extended from day 8 to hatching and older embryos cannot be stages in ovo. Second, the stages of Hamberger and Hamilton's series from 11 days on represents one day intervals which would not be sufficiently differential for this study. However, external characteristics as outlined by Hamberger and Hamilton were subjectively compared, where possible, with the embryos in this study in

order to ascertain relative developmental normalcy.

D. Statistical Treatment of the Data

The mean rate of beak clapping was determined for the A.M. readings, the P.M. readings, and the total day readings. An analysis of variance was computed through the use of the Fisher Test by comparing a) one day vs. another day or group of days, b) A.M. vs. P.M., and c) embryo vs. embryo to determine error or animal variance.

CHAPTER IV

FINDINGS

Of the 48 White Leghorn eggs incubated, 23 were included in this study. The remaining 25 eggs were excluded from statistical evaluation due to the following reasons: 1) the egg was determined non-fertile at day 7, 2) the embryo died at some time during development after the window was established, 3) beak movement was not visible or was undeterminable at one or more observation periods, or 4) the abnormal appearance of the chick within a 48 hour post-hatching period, (Table No. 1).

The mean rate of beak clapping per 15 seconds for the A.M., P.M., and day total was as indicated in Table No. 2. The observations made on day 8 and the morning of day 9 demonstrated a lack of sufficient development of the beak to define movement. By the evening observation of day 9, twelve embryos were sufficiently developed so that beak movement could be recorded. From day 10 to day 19, beak movement was determinable for all 23 embryos for all A.M. and P.M. observations. It was found that 39% of the chick embryos initiated bill clapping on day 9 with the remainder initiating such movements on day 10, (Appendix). Hatching occurred from

TABLE No. 1

DESCRIPTION OF SAMPLE

Status of the Embryo	Number
1. Non-fertile	18*
2. Died Post-Operative	5
3. Normal but Beak Movement Undeterminable	2
4. Post-Hatching Abnormalities	0
5. Normal and Beak Movement Determinable	23
Total	48

* Poor vitality of sample was attributed in part to subzero outside winter temperatures three days prior to and including the day of collection.

TABLE No. 2

MEAN RATE OF BEAK CLAPPING PER 15 SECOND PERIOD

A.M., P.M., DAY TOTAL

Day of Incubation	A.M. Mean	P.M. Mean	Day Total Mean	1 S.D. for Day Total
8	*	*	*	--
9	*	1.3	1.3	± 1.1
10	1.4	1.9	1.7	± 1.2
11	3.7	3.6	3.6	± 1.7
12	3.1	3.2	3.2	± 1.8
13	4.3	3.0	3.6	± 1.8
14	2.1	2.8	2.5	± 1.8
15	1.8	2.3	2.0	± 1.6
16	2.6	1.6	2.1	± 1.9
17	1.6	1.4	1.5	± 1.8
18	1.6	2.0	1.8	± 2.2
19	2.0	1.5	1.7	± 1.9
20	1.8	2.9	2.1	± 2.0

* Beak not sufficiently developed to define movement

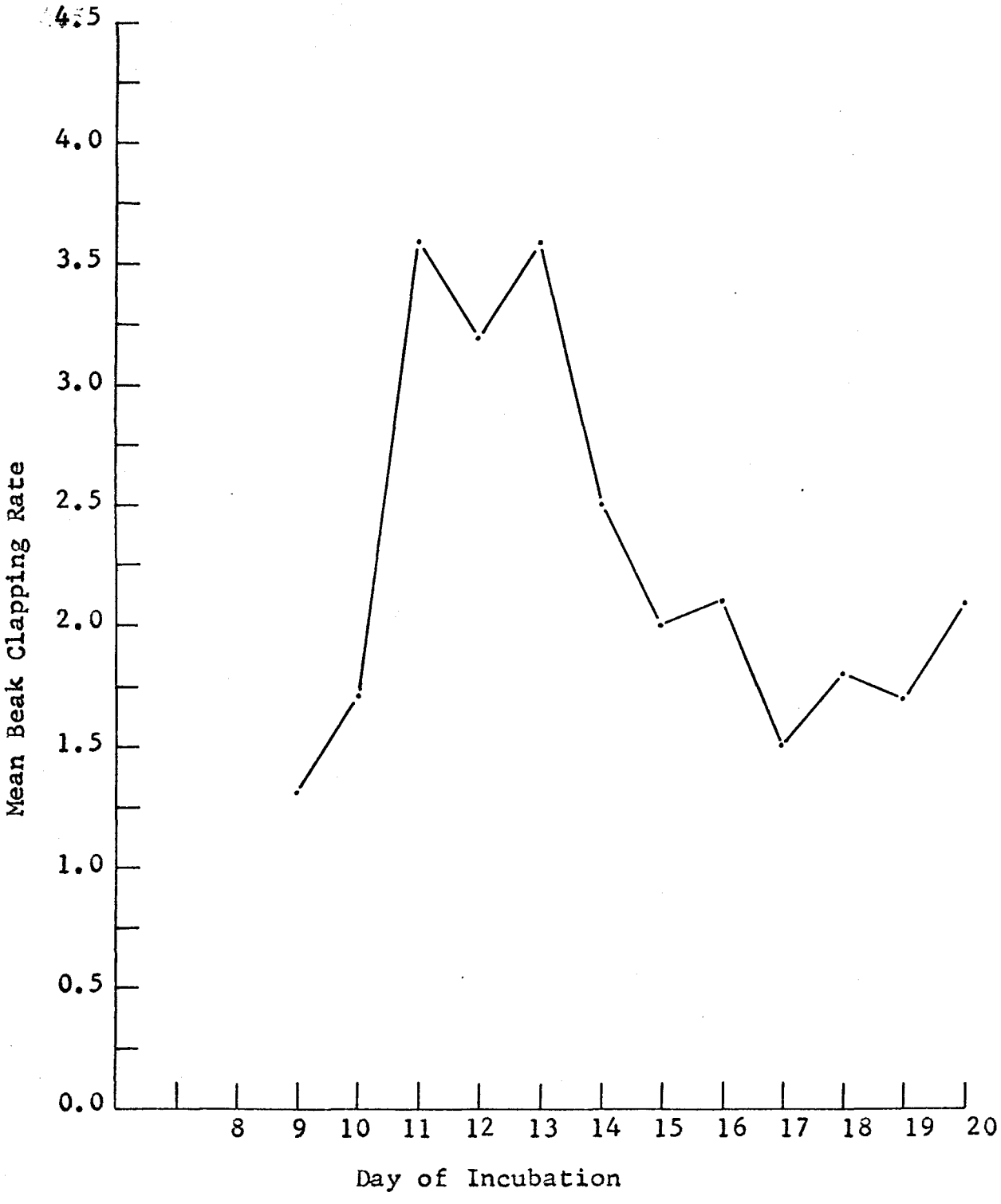

Fig. 5 Day total mean rate of beak clapping per 15 seconds.

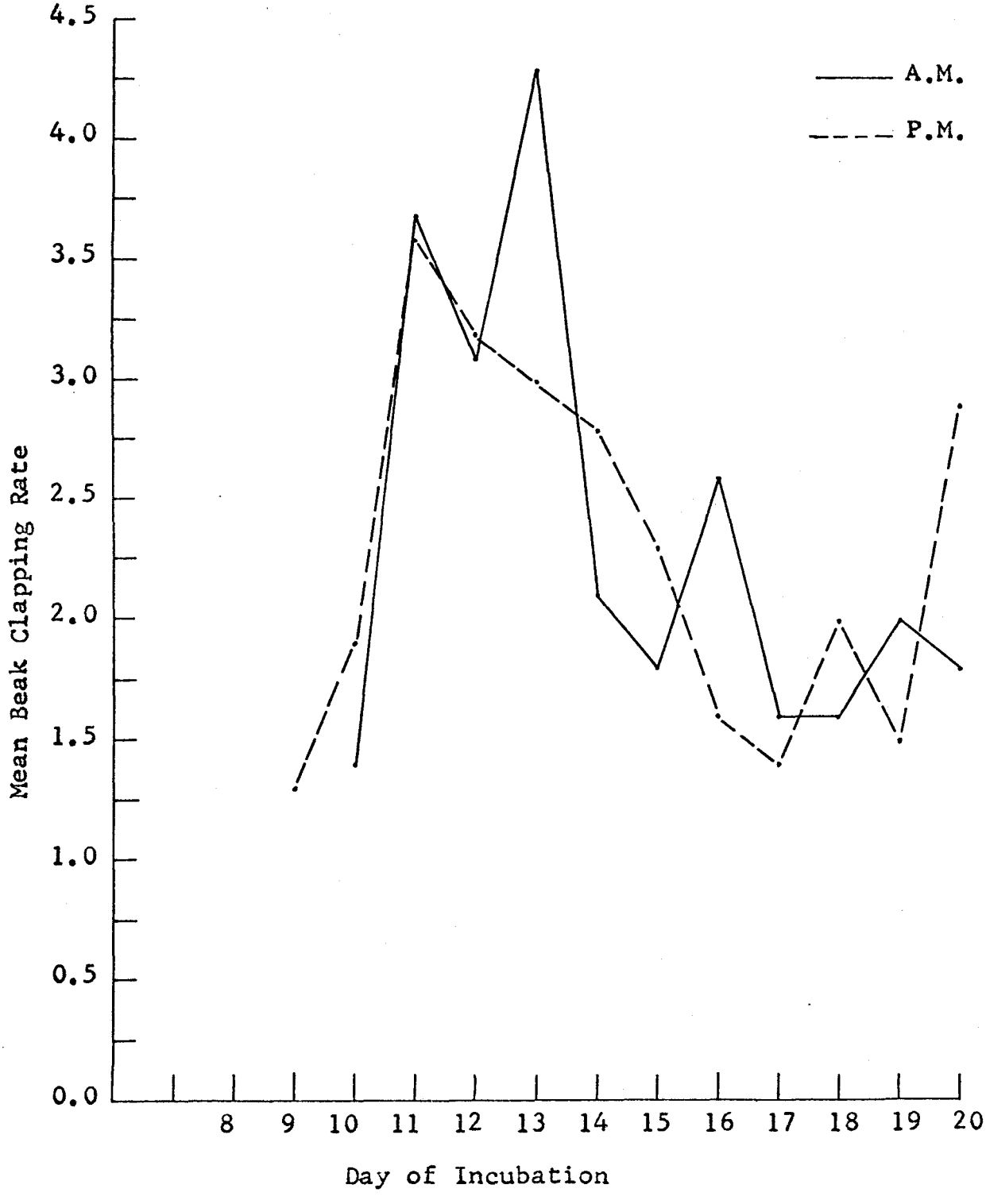

Fig. 6 A.M. and P.M. mean rates of beak clapping per 15 seconds.

TABLE No. 3

ANALYSIS OF VARIANCE IN THE MEAN RATE OF BEAK CLAPPING

Sources of Variation	DF	SS	MS	F
A. Days				
11 vs 12	1	5.26	5.26	1.48
11,12 vs 13	1	1.45	1.45	0.41
15 vs 16	1	0.10	0.10	0.03
18 vs 19	1	0.01	0.01	0.003
15,16 vs 17	1	9.42	9.42	2.65
15,16,17 vs 18,19	1	1.10	1.10	0.31
10 vs 15,16,17, 18,19	1	1.28	1.28	0.36
14 vs 10,15,16,17, 18,19	1	16.77	16.77	4.71*
11,12,13 vs 10,14,15,16, 17,18,19	1	236.97	236.97	66.57**
Total	9	272.36	30.26	8.49**
B. A.M. vs P.M.	230	693.50	3.02	0.85
C. Embryo vs Embryo	220	783.04	3.56	
Total	459	1748.90		

* .05 > P > .01

** P < .001

day 20 to day 21 with all embryos hatching within a 24 hour period, (Appendix). It was noted that a sharp increase in the day total mean beak clapping rate occurred on days 11, 12, and 13 with somewhat of a reduction on day 14 and an apparant leveling off from day 15 to hatching, (Fig. 5). A.M. and P.M. mean beak clapping rates generally followed this same pattern, (Fig. 6).

The analysis of variance in the mean rate of beak clapping was computed utilizing data from day 10 to day 19 inclusive. Days 9, 20, and 21 were excluded from statistical evaluation because of incomplete data due to the lack of sufficient beak development or hatching. It was noted that day 14 was significantly different from days 10,15,16,17,18, and 19 ($.05 > P > .01$); and that days 11,12, and 13 were highly significant in their difference from days 10,14,15,16,17,18, and 19 ($P < .001$), Table No. 3. Such were the only areas of statistical difference upon computing all the possible sources of variation.

In the comparison of the A.M. and P.M. mean rates of beak clapping, it was found that no significant difference resulted.

CHAPTER V

DISCUSSION

A high frequency of the mean rate of beak clapping of the White Leghorn chick embryo was maintained throughout embryonic development from day 9 to hatching. An analysis of variance indicated that day 14 was significantly different from days 10,15,16,17,18, and 19 ($.05 > P > .01$); and that days 11,12, and 13 were highly significant in their difference from days 10,14,15,16,17,18, and 19 ($P < .001$). In the comparison of days as sources of experimental variation, it was found that the high mean beak clapping rate on days 11,12, and 13 was responsible for 87% of the total daily variance. Such an increase in the mean beak clapping rate was evidence for increased quadrate-quadratojugal articular movement during the same period. Therefore, increased articular movement was seen to occur on days 11,12 and 13. Increased articular movement was also noted on day 14 which was significantly different from days 10,15,16,17,18, and 19.

It should be noted that in comparing A.M. rates with P.M. rates that no significant difference resulted. Therefore, the 12 hour on - 12 hour off incubator lighting cycle did not

affect the mean beak clapping rate.

It was reported by Lillie (1927) that all embryonic structures of the quadrate-quadratojugal articulation are present and are ready for maturation at the begining of the tenth day. In addition, Hamberger and Hamilton's (1951) description of the development of the visceral arches is compatible and complements Lillie's description. The present investigation has found that there is peak articular movement on days 11,12,13,and 14. Therefore, an apparant relation exists between the histologic appearance of the articular components and the subsequent immediate increase of articular movements. In addition, such increased articular movements may be necessary for the normal anatomic and functional development of the quadrate-quadratojugal articulation, (Drachman and Sokoloff, 1966).

This investigation differed from Kuo (1932) in that bill clapping was initiated in 39% of the embryos on day 9 with the remaining embryos initiating such movements on day 10. Kuo's findings--which stated that bill clapping first occurred at day 9 in 14.6% of the embryos, at day 10 in 39.1%, and at days 11 and 12 in the remaining embryos--probably differed due to the fact that a) he included mixed Chinese breeds in his sample as well as White Leghorns, and b) his method of observation differed considerably from those used in the

present study.

Although the beak clapping rate was the only embryonic movement from which data was prepared, other embryonic motility was also observed throughout incubation. Such observations included the mechanical factors leading to hatching and the effect of embryonic malpositions as discussed by Kuo (1932); and general somatic motility, whether spontaneous or coordinated, as discussed by Hamberger et al., (1963,1965,1966,1967). Observations made by Kuo and Hamberger were as observed by this investigator, and such embryonic positioning and generalized motility made beak observation, at times, extremely difficult. It should be noted that whereas general embryonic activity rises steadily from day 3½ to day 13 and is maintained to day 17 (Hamberger et al. 1965), this investigation found that a significantly high beak clapping rate is only present on days 11,12,13, and 14. Therefore, it can be seen that while general embryonic activity remains at a high level, beak clapping decreases sharply from day 14.

Hamberger et al. (1967) reported higher mean rates of beak clapping for days 15,16,17,18, and 19 than are presently reported in this investigation. It should be remembered, however, that in Hamberger's investigation, if a beak was buried in the yolk sac or covered by the wing, a small opening was made in the membranes and the beak exposed. Recordings

made subsequent to the opening of the membranes cannot be as accurate as those recordings made without any such immediate disturbance of the embryonic structures.

A cooler incubation temperature of 36° C. had been used in this investigation as opposed to the so called optimum temperature of 37.8° C. as described by Hamilton (1952). It was found that increased viability of the embryos resulted due to the fact that the incubator used in this investigation was not a forced air incubator.

It should be noted that a 12 hour on - 12 hour off cycle of the incubator lights was used so as not to alter the normal circadian cycle of the biologic system. Due to the investigations made by Tamimie et al. (1967) and Gimeno (1967) on the effect of light on the embryonic development of the chick embryo, only sufficient light was used such that an adequate observation of the embryo in ovo was possible. It should be noted, however, that Kuo (1932) stated that the embryo's response to light did not occur until the end of the seventeenth day, and that such light may only cause the embryo to open or close its eyes without involving other body parts. Whatever the effect of light on the embryos, lighting was kept to a minimum in this investigation and no ill effects were noted in the test embryos.

Kuo's (1932) shell cap method of embryonic observation was criticized by Windel et al. (1934) because Windel was

unable to render the inner shell membrane transparent with vaseline to observe fine movements. Therefore, such a technique was not employed in this investigation. The use of the window technique, with plastic kitchen wrap covering the opening, afforded this investigator an excellent opportunity to observe the intricate embryonic development. It was likely that this procedure also influenced the pressure balance between shell, air chamber, and embryo as discussed by Kovach (1968). It should be noted, however, that embryonic development progressed normally in 83% of the embryos opened (5 embryos died at some time after the window was established), and that 100% of the embryos that hatched appeared normal during the 48 hour post-hatching period.

The chick embryo was selected as the test animal in this investigation for the following reasons: 1) the chick embryo quadrate-quadratojugal articulation is a bilateral synovial lined diarthrodial joint with an interposing disc as is the human temporal mandibular joint, 2) the chick embryo is a vertebrate and can, therefore, be compared to other vertebrates, and 3) the chick is especially adapted to embryonic studies due to the ready accessibility of the embryo just beneath the protective shell.

CHAPTER VI

SUMMARY AND CONCLUSIONS

It was the purpose of this investigation to note the normal operant movement of the White Leghorn chick embryo quadrate-quadratojugal articulation. Evidence of articular movement was noted by determining the mean rate of beak clapping. An accurate reproducible methodology was proposed for the observation of beak movement while maintaining in vivo conditions. The mean rate of beak clapping was determined for A.M., P.M., and day total observations of 23 embryos. A theory was proposed for the relation between articular development and articular function.

It was found in comparing the day total mean rate of beak clapping that day 14 was significantly different from days 10, 15,16,17,18, and 19 ($.05 > P > .01$); and that days 11,12, and 13 were highly significant in their difference from days 10,14, 15,16,17,18, and 19 ($P < .001$). Such an increase in the mean beak clapping rates on days 11,12,13, and 14 was evidence for increased articular movement during the same period.

It was found that embryonic structures of the quadrate-quadratojugal articulation were present and were ready for

maturation at the begining of the tenth day. The histologic appearance of the embryonic structures of the quadrate-quadratojugal articulation was followed by an immediate and significant increase in articular movements. Increased articular movement occurred for four days following the appearance of the articulation, after which such articular movement was reduced sharply and leveled off for the remaining embryonic development.

It was found that in comparing A.M. and P.M. rates of beak clapping that no significant difference resulted. Therefore, the 12 hour on - 12 hour off incubator lighting cycle did not affect the mean beak clapping rate.

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APPENDIX

Rate of Beak Clapping Per 15 Second Period

Embryo Number		8d	9d	10d	11d	12d	13d	14d	15d	16d	17d	18d	19d	20d	21d
1	AM	*	*	0	1	0	5	3	5	1	2	0	2	2	
	PM	*	*	1	4	3	3	1	0	2	3	1	2	**	
2	AM	*	*	3	6	2	2	4	6	2	2	2	2	0	**
	PM	*	*	2	1	0	4	2	2	0	0	6	3	2	
3	AM	*	*	0	1	3	6	5	5	1	2	2	1	0	**
	PM	*	*	0	2	4	3	0	2	1	0	0	0	4	
4	AM	*	*	3	4	6	7	3	3	8	0	2	2	4	
	PM	*	*	3	5	3	7	8	3	5	1	0	0	**	
7	AM	*	*	1	4	6	5	1	2	8	1	2	0	**	
	PM	*	1	1	6	4	1	3	3	1	2	2	3		
9	AM	*	*	0	4	0	3	5	0	2	2	3	4	0	
	PM	*	0	2	2	3	3	1	2	3	4	0	0	**	
10	AM	*	*	3	4	2	5	3	6	2	1	5	0	2	
	PM	*	0	1	4	3	1	3	2	0	0	8	0	**	
12	AM	*	*	1	3	0	1	2	0	0	0	0	1	0	
	PM	*	*	1	4	3	1	2	2	0	3	5	4	**	
13	AM	*	*	0	3	4	4	0	2	1	1	2	0	3	
	PM	*	*	3	4	2	4	1	5	2	1	4	0	**	

APPENDIX

Rate of Beak Clapping Per 15 Second Period

Embryo Number		8d	9d	10d	11d	12d	13d	14d	15d	16d	17d	18d	19d	20d	21d
14	AM	*	*	2	5	6	8	2	2	4	5	0	1	3	
	PM	*	4	2	3	5	2	4	5	2	5	0	5	**	
15	AM	*	*	0	3	6	5	2	0	3	6	0	0	5	
	PM	*	2	2	7	4	4	2	3	2	0	2	0	**	
16	AM	*	*	4	2	1	2	3	1	1	0	1	1	1	**
	PM	*	2	2	3	2	3	2	2	2	6	0	3	0	
17	AM	*	*	2	4	3	5	2	0	2	7	0	7	**	
	PM	*	1	2	1	2	3	1	1	2	0	2	2		
18	AM	*	*	0	5	3	5	1	1	5	2	0	5	0	
	PM	*	1	3	1	4	6	7	2	0	0	2	4	**	
19	AM	*	*	2	4	5	4	2	0	6	0	1	2	2	**
	PM	*	1	1	4	2	2	2	3	2	0	0	5	5	
21	AM	*	*	1	3	6	4	3	2	3	0	2	2	0	**
	PM	*	1	3	6	6	4	2	4	1	1	7	0	4	
22	AM	*	*	3	2	4	4	3	1	3	0	2	0	**	
	PM	*	2	3	3	5	3	4	1	0	3	0	0		
23	AM	*	*	0	3	1	4	0	3	2	0	0	6	5	**
	PM	*	*	0	2	6	3	2	3	3	0	3	0	1	

APPENDIX

Rate of Beak Clapping Per 15 Second Period

Embryo Number		8d	9d	10d	11d	12d	13d	14d	15d	16d	17d	18d	19d	20d	21d
25	AM	*	*	3	4	2	5	1	0	1	1	1	3	**	
	PM	*	*	3	6	4	0	5	2	1	2	0	0		
26	AM	*	*	3	3	4	2	0	1	2	0	1	0	1	
	PM	*	*	4	4	2	2	1	2	3	1	0	0	**	
27	AM	*	*	1	5	2	3	1	0	0	1	1	1	0	**
	PM	*	*	1	2	0	3	2	2	1	0	3	0	7	
28	AM	*	*	0	4	4	2	1	0	1	1	1	2	3	
	PM	*	0	3	1	1	2	2	1	0	1	0	0	**	
30	AM	*	*	0	7	2	7	2	1	2	3	8	4	3	**
	PM	*	*	1	8	5	4	7	1	4	0	0	3	0	

* Beak not Sufficiently Developed to Define Movement
** Embryo Hatched

APPROVAL SHEET

The thesis submitted by James A. Coglianese, B.S., D.D.S. has been read and approved by members of the Department of Oral Biology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 1, 1970
Date

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Signature of Advisor